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Research Article

PHYSIOLOGICAL AND BIOCHEMICAL STUDY OF FOUR NEW MAIZE LINES (*ZEA MAYS* L.) IN DALOA, COTE D'IVOIRE

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ABSTRACT

Maize (*Zea mays* L.) is a cereal belonging to the Poaceae family that adapts to a variety of ecological conditions. However, increasing climate change is leading to numerous abiotic and biotic stresses that are reducing its production. Faced with these challenges, it is becoming essential to develop new maize varieties with characteristics that can adapt to changing climatic conditions. The study focused on the physiological and biochemical characterisation of four maize lines derived from the EV8728 variety irradiated with gamma radiation after 6 generations of self-fertilisation. These lines were compared with the unirradiated parent variety EV8728. The results showed a significant effect on all the parameters assessed. At physiological level, chlorophyll (a), (b) and total contents were higher in the non-irradiated EV8728 variety and line S03. Biochemically, phenolic compound and total sugar levels were highest in lines S20 and S24. In terms of enzymatic activity, lines S03 and S76A showed higher levels of polyphenol oxidase (PPO). Lines S20 and S24 showed higher levels of peroxidase (POD) and line S76A accumulated more catalase (CAT) than the witness.These biochemical variations probably reflect distinct adaptation and resistance mechanisms within these lines.

Keywords: Maize, Line, Climate change, Biochemical, Gamma radiation.

INTRODUCTION

Maize (*Zea mays* L.) is an annual herbaceous plant belonging to the Poaceae family, also known as grasses. This tropical cereal adapts to a wide range of ecological zones (Bânziger & Diallo, 2001). As one of the world's main cereal crops, maize plays an essential role in food security (FAO, 2020). Global maize production stands at around 1.2 billion tonnes, with an estimated area of 20 million ha (FAOSTAT, 2022). After rice, maize production in all rural areas of Côte d'Ivoire has risen from 1,025,000 tonnes in 2017 to 1.2 million tonnes in 2020, covering 56 thousand hectares (FAOSTAT, 2022). In developed countries, maize is mainly used for animal feed. In developing countries, on the other hand, maize is mainly used for human consumption (Doffangui, 1997).Apart from these food uses, maize is also put to good use in industry, notably for the manufacture of infant flour, beer production and the formulation of livestock feed (Yapi, 2017). Despite these multiple uses and interests, maize cultivation in Côte d'Ivoire generally shows a low level of productivity due to the loss of fertility of cultivable land, the use of traditional varieties with low potential, the genetic degeneration of cultivated varieties and, above all, the impact of climate change (Lobell *et al*., 2011). To deal with the various constraints on maize growing, new improved varieties need to be developed. In this context, research programmes aim to develop maize varieties that are better adapted to local soil and climate requirements. Obtaining new, improved maize varieties necessarily involves crossing selected lines. Mastery of these parent lines is therefore essential if genetic improvement programmes are to be effective. In particular, this requires a good knowledge of their metabolic profile, which is an indicator of their nutritional quality and resistance to environmental factors. With this in mind, the general objective of this work is to understand the agronomic behaviour of new yellow maize lines (*Zea mays* L.) in Daloa (Côte d'Ivoire).

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MATERIAL AND METHODS

Study site

The research work was carried out on the experimental plot at the Université Jean Lorougnon Guédé, located in Daloa city, central-western Côte d'Ivoire. Daloa's geographical coordinates are 6°53'38 north latitude and 6°27'0 west longitude. The city is an integral part of the Haut-Sassandra administrative region. Daloa lies in an area once covered by dense forest, which has now given way to a variety of cash crops. The region's soils are ferralitic and the climate is tropical. The average temperature is 27.5°C, with annual rainfall varying between 1,000 and 1,500 mm. The study site soils are predominantly ferralitic (Ayolié *et al*., 2022).

Plant material

The plant material used in this study consisted of leaves from three-week-old maize plants (Figure 1). More precisely, these were the third leaves from the apex of the plants. The maize plants were obtained by germinating seeds from four different lines and a witness variety EV8728 (Table I).

Table 1. List of lines and varieties of EV8728 maize.

LIGNEES	CODES
LIGNE 1	S03
LIGNE 2	S20
LIGNE 3	S24
LIGNE 4	S76A
WITNESS EV 8728	T0

Figure 1. Maize Plants.

MATERIALS AND METHODS

Experimental set-up

The experiment was set up using a simple design consisting of 5 lines (L1, L2, L3, L4 and LT). Each of these 5 lines corresponds to a different maize line, with 5 pots per line. Two maize seeds were sown in each pot. The lines were spaced 1 m apart, while the pots in the same line were 0.5 m apart.

Sampling and leaves conservation

Sampling consisted of removing the third leaf from the top of three-week-old maize plants using scissors. These samples were taken simultaneously from all the plants. Immediately after collection, the leaf samples were placed in ice to keep them at a low temperature. The samples were then transported to the laboratory where they were stored at a temperature of -29°C in a refrigerator.

Extraction and components assaying

Physiological components

Only physiologicals components analysed in this study were chlorophyll pigments and carotenoids. These leaf pigments were extracted and assayed using the method described by Lichtenthaler in 1987. 0.1 g of fresh maize leaves were immersed in 10 mL of 95% acetone, then kept in the dark at 4°C for 48 hours. Next, 3 mL of supernatant was collected for optical density (OD) readings at 663 nm and 647 nm for chlorophyll and 470 nm for carotenoid. Concentrations were calculated using the following formular :

Chlorophyll a (µg/g MF) = 12.25*DO663 - 2.79*DO647 *(V/1000*m)

Chlorophyll b (µg/g MF) = 21.5*DO647 - 5.10*DO663 *(V/1000*m)

Total chlorophyll (µg/g MF) = 7.15*DO663 + 18.71*DO647*(V/1000*m)

Carotenoids (µg/g MF) = (1000*DO470 - 182*chl a - 85.02*chl b)/198

MF: fresh matter; **m**: mass and **V**: final volume

Biochemical components

Extraction and phenolic compounds assaying

Phenolic compounds were extracted according to Kouakou (2009) method.500 mg of leaves were placed in 10 mL of methanol (96%) and then incubated in the dark for 10 h at 4°C. The leaves were then centrifuged at 5000 rpm for 10 min. Phenolic compounds were determined according to Singh (2000) method. To do this, 0.5 mL of 5 N folin calcium reagent was added to 0.9 mL of distilled water, followed by the addition of 0.1 mL of phenolic extract. After shaking, 1.5 mL of 17% sodium carbonate was added to the solution and incubated for 30 minutes. The colour intensity was read at 765 nm. The total phenols rate was determined using a calibration curve performed with different concentrations of a gallic acid stock solution (200 µg/mL).

Extraction and total sugars determination

Total sugars were extracted in the same way as phenolic compounds. The assay was carried out using Dubois *et al*. (1956) method. Thus, 0.2 mL of 5% phenol and 0.2 mL of phenolic extract were taken and placed in a test tube, then made up to 1 mL with distilled water. Next, 1 mL of concentrated sulphuric acid (97%) was added. The solution was incubated for 5 min in a boiling bath, then cooled in the dark for 30 min. The intensity was measured with a spectrophotometer at 490 nm. The total sugar content was determined using a calibration line (0.01 to 0.1 mg.mL-1) constructed from a glucose solution (1mg.mL-1).

Extraction and proline assay

Proline was extracted and assayed using the method of Dreir & Goring (1974). 100 mg of fresh leaves were crushed in 3 ml of 40% methanol and then heated in a water bath at 85°C for 30 minutes. After cooling and centrifugation at 4000 rpm for 10 min, 1 mL of the supernatant was removed. Next, 1 mL glacial acetic acid, 25 mg ninhydrin and 1 mL of mixture (I) containing (120 mL distilled water, 300 mL acetic acid (CH3COOH) and 80 mL phosphoric acid (H3PO4)) were added. The mixture was homogenised and boiled at 100°C for 45 minutes until the red colour changed. After cooling, 5 mL of toluene was added to the solution, which was then stirred and left to stand for 30 min.The optical density (OD) is read using a spectrophotometer at 528 nm.

Extraction and enzymatic proteins assay

Enzyme protein extraction

Enzymes were extracted cold (4°C) by grinding 500 mg of fresh leaf in 5 mL of 0.1 M phosphate buffer in the presence of 0.05 g of PVP. During grinding, 0.1 mL of a solution composed of 5% polyethylene glycol 6000 (PEG 6000), 0.25% sodium thiosulphate, 15% glycerol, 1 mM EDTA and 15mM mercaptoethanol was added. After centrifugation at 5000 rpm for 20 min, the supernatant obtained represented the crude enzyme extract.

Polyphenoloxidase assay

Polyphenoloxidases (PPO) were assayed according to Zhou *et al* (2003) method. A reaction volume of 3 mL consisting of 0.2 mL enzyme extract, 1 mL pyrocatechol and 1.8 mL 0.1 M phosphate citrate buffer pH 6.5 was used for the assay. The oxidation was read using a spectrophotometer at a wavelength of 500 nm. The molar extinction coefficient was 1400 M-1cm-1.

Peroxidase assay

Peroxidase activity (POD) was determined according to Santimone (1973) method. Sodium phosphate (0.1 M) at pH 7.5 was used as the phosphate buffer. During the assay, 0.1 mL of the enzyme extract was taken and placed in test tubes. To this extract, 2.9 mL of a substrate consisting of a 10-2 M solution of guaiacol and 10-2 M (v/v) hydrogen peroxide (H2O2) was added. The reaction mixture was stirred and then incubated in the dark for 10 min. The oxidation of guaiacol was read using a spectrophotometer at 470 nm. POD activity is expressed as a molar extinction coefficient of 26.6 mM-1 cm-1.

Phenylalanine ammonia-lyase and tyrosine ammonia-lyase determination

Phenylalanine ammonia-lyase (PAL) and tyrosine ammonia-lyase (TAL) determination was carried out according to Regnier (1994) method. The base buffer used was 0.2 M sodium borate at pH 8.8. The assay was carried out using 0.1 mL of enzyme extract, 1 mL of 0.1 M

phenylalanine for PAL or 0.1 M tyrosine for TAL and 1.9 mL of 0.2 M sodium borate buffer at pH 8.8. The reaction mixture was incubated at room temperature for 30 min and the activities of PAL and TAL were read spectrophotometrically at 290 nm. The molar extinction coefficient of the cinnamic acid formed was 19600 M-1cm-1 and that of p-coumaric acid was 17600 M-1cm-1.

Ascorbate peroxidase and catalase determination

Catalase (CAT) and ascorbate peroxidase (APX) activities were determined using the method of Zhou *et al*. (2003). The APX assay was carried out using 3 mL of reaction volume comprising 0.1 mL of enzyme extract and 2.9 mL of ascorbic acid solution. APX activity was read using a spectrophotometer at 290 nm. The CAT assay was carried out using the same reaction medium, including 0.1 mL enzyme extract, 1 mL H2O2 and 1.9 mL Tris-HCl buffer. Catalase activity was read using a spectrophotometer at 240 nm. The molar extinction coefficient was 36.10-6M-1.cm-1.

Statistical analysis

Data relating to the various physiological and biochemical parameters measured for each line were processed using IBM SPSS STATISTICS software, version 22. An analysis of variance (ANOVA) was performed on the data. When significant differences were identified at the 5% threshold, Tukey's post-hoc test (HSD) was applied to classify the means of the different groups.

RESULTS AND DISCUSSION

The results relating to changes in leaf pigments determined during this experiment are presented in Table II. The variation in chlorophyll content between the different lines was significant ($P < 0.05$). However, there was no significant difference in carotenoid accumulation between the lines studied. Line S03 accumulated more chlorophyll a (CHLa) than the other lines and the witness. The lowest CHLa content was obtained in line S76A. On the other hand, for chlorophyll b (CHLb) and total chlorophyll (CHLt), the Witness recorded the highest levels compared with the other lines.

Table 2. Leaf pigment content in maize lines.

	Variables (µg /mL)			
LIGNEES	CHLa	CHLb	CHLt	CARO
STO	0.45 ± 0.20^{b}	0.83 ± 0.65^{a}	1.28 ± 0.66^{a}	9.43 ± 2.69^{a}
S03	0.95 ± 0.28^{a}	0.24 ± 0.15^{b}	0.92 ± 0.16^{ab}	7.53 ± 0.44^{a}
S20	0.29 ± 0.19^{c}	0.35 ± 0.39^{b}	0.64 ± 0.25^{b}	7.95 ± 1.88^{a}
S24	0.29 ± 0.05^{c}	0.34 ± 0.39^{b}	0.63 ± 0.38^{b}	6.77 ± 1.69^{a}
S76A	0.20 ± 0.08^{c}	0.33 ± 0.21^{b}	0.52 ± 0.14^{b}	7.24 ± 1.35^{a}
F	16.453	6.005	3.972	1.226
P	0.001	0.025	0.013	0.325

In the same column, averages followed by the same letter are not significantly different at the 5% level.

Table 3. Biochemical compound content in the maize lines and witness leaves.

	Variables		
LIGNEE	COMP PHE (µg /g MF)	SUGARS (µg /g MF)	PROL (mM /g MF)
TEMOIN	0.67 ± 0.076^{c}	6.93 ± 1.48^{c}	0.07 ± 0.04^{a}
S03	0.74 ± 0.14^{c}	7.79 ± 2.14^{bc}	0.07 ± 0.03^{a}
S20	1.17 ± 1.22^{a}	8.95 ± 1.75^{b}	0.08 ± 0.01^{a}
S24	0.86 ± 0.27^{b}	10.68 ± 3.44^{a}	0.09 ± 0.019^{a}
S76A	0.86 ± 0.07^{b}	10.47 ± 1.72^{a}	0.09 ± 0.02^{a}
F	7.691	4.286	1.004
P	0.005	0.027	0.424

Means in the same column followed by the same letter are not significantly different at 5%.

The biochemical compounds accumulated by the maize lines studied are shown in Table III. With the exception of proline content, the levels of phenolic compounds (COMP PHE) and total sugars showed a significant effect ($P< 0.05$) depending on the lines studied. Line S20 accumulated more phenolic compounds in its leaves, followed by lines S24 and S76A, compared with the witness, which also had the lowest phenolic compound content. In terms of total sugars, lines S24 and S76A accumulated more sugars than the other lines and the witness. However, the witness still had the lowest total sugar content.

Leaves enzyme content variation from the different lines is shown in Table IV. This variation according to the lines revealed a significant effect ($P < 0.05$) for the enzymes phenyalamine-ammonia-lyase (PAL), polyphenoloxidase (PPO), peroxidase (POD) and catalase (CAT). However, this effect was not significant for tyrosine

ammonia lyase (TAL) and ascorbate peroxidase (APX). PAL activity in the witness was about three times higher than in the lines studied. On the other hand, PPO activity was lower in the witness and in line S24. Lines S03 and S76A accumulated more PPO. In addition, lines S20 and S24 recorded higher POD enzyme activities than the witness. However, in terms of catalase (CAT) activity, line S76A recorded the highest activity compared with the witness and the other lines. Moreover, catalase activity was lowest in the cowntrol.

Table 4. Enzyme content in maize leaves from the different lines and the witness.

	Variables : TAL, PAL, PPO et POD (µmol/min/g MF) ; CAT et APX (mmol/min/g MF)					
LIG	TAL	PAL	PPO	POD	CAT	APX
ST0	3.90 ± 1.25^{a}	0.84 ± 0.47^{a}	5.75 ± 3.38^{b}	361.09 ± 178.89^{b}	1586.01 ± 322.23^{b}	2277.82 ± 299.81^{a}
S03	3.92 ± 1.60^{a}	0.27 ± 0.20^{b}	10.47 ± 0.98^{a}	351.88 ± 105.01^{b}	2202.98 ± 394.56^{ab}	2216.07 ± 227.24^{a}
S20	4.63 ± 1.79^{a}	0.27 ± 0.08^{b}	7.91 ± 1.71^{ab}	623.30 ± 176.99^{a}	2271.7 ± 562.13^{ab}	2328.12 ± 118.22^{a}
S24	4.09 ± 0.65^{a}	0.30 ± 0.08^{b}	5.35 ± 1.58^{b}	604.89 ± 143.03^{a}	2244.26 ± 461.97^{ab}	2205.36 ± 215.94^{a}
S76A	3.31 ± 0.48^{a}	0.32 ± 0.09^{b}	10.15 ± 3.05^{a}	470.86 ± 105.76^{ab}	2407.11 ± 492.41^{a}	2095.53 ± 185.59^{a}
F	0.836	6.47	6.33	5.729	2.986	0.972
P	0.515	0.001	0.001	0.002	0.038	0.441

DISCUSSION

In order to study the physiological and biochemical behaviour of certain maize lines, analyses were carried out on several parameters such as : the content of leaf pigments, phenolic compounds, proline, total sugars and enzymes such as tyrosine ammonia lyase (TAL), phenylalanine ammonia lyase (PAL), polyphenoloxidase (PPO), peroxidase (POD), catalase (CAT) and acrobate peroxidase (APX). The results show significant differences between the lines studied for all the parameters analysed. Physiologically, total chlorophyll (chl t) and chlorophyll b (chl b) levels were higher in the control than in the other lines. Chlorophyll a (chl a) content was higher in line S03. Chlorophyll concentration varies according to the maize lines studied. This difference is thought to be linked to the photosynthetic activity of each line. High chlorophyll content generally indicates good photosynthetic activity, which translates into better plant growth and development. According to Intani & Laurentius (2022), chlorophyll, an essential photosynthetic pigment, also acts as a marker of plant health. In addition, studies by Bougdad (2015) have shown that water stress affects alfalfa plants by causing a reduction in leaf cell size. This results in an increase in chlorophyll content in the leaves. Similarly, work by Nguinambaye *et al* (2020) on duckweed plants subjected to a stressed water regime revealed a high concentration of chlorophyll in the leaves. This would be linked to increased photosynthesis as a mechanism for adapting these plants to water stress.

In terms of biochemical parameters, the results revealed significant differences in phenolic compound and total sugar content. Line S20 accumulated a greater quantity of phenolic compounds than the other lines. Phenolic compounds are secondary metabolites produced by plants mainly for growth, development and protection against various stresses. The high phenolic compound content observed in line S20 could therefore be explained by the line's greater ability to defend itself against various stresses. This suggests that line S20 has better resistance and adaptation abilities thanks to its biochemical profile rich in protective phenolic compounds. Phenolic compounds play an essential role in the plant's interactions with biotic and abiotic stresses (Sambangi, 2022). Other studies, carried out by Usha & Jyothsna (2010) and Pratyusha & Usha (2016) on infected rice and groundnut plants, also observed an increase in the content of phenolic compounds. According to these authors, this accumulation of phenolic compounds in stressed or infected plants is an important defence mechanism. These secondary metabolites act as a physico-chemical barrier to the feeding of insect pests and other pathogens. As a result, line S20 seems to have all the biochemical assets needed to cope with any problems linked to biotic or abiotic stresses. In terms of total sugars, line S24 was found to have the highest content compared with the other lines studied. This high accumulation of sugars in line S24 could be explained by the fact that the plants were grown in buckets, which may have induced high metabolic activity. In fact, the water stress associated with this method of cultivation in pots has probably prompted the S24 line to adapt in order to survive. According to several authors, such as Lisar *et al* (2012) and Lipiec *et al* (2013), the accumulation of sugars under drought conditions helps plants to maintain the stability of their membranes, protect cellular integrity and preserve the functioning of their proteins. Thus, the high concentration of total sugars observed in the S24 line suggests that this line has better capacities to adapt and maintain its physiological functioning under stress.

The activities of the PAL, PPO, POD and CAT enzymes were found to be significantly different between the lines studied. Polyphenoloxidase (PPO) activity was higher in the S03 and S76A lines. PPO plays an important signalling role in the defence of plants against biotic and abiotic stresses. The higher PPO activity observed in these two lines indicates their greater capacity for tolerance and resistance to aggression. These results are in line with the work of Ngazee *et al* (2012) who showed that potato varieties with increased PPO activity were also more tolerant to the soft rot disease caused by pathogenic bacteria.The greater accumulation of peroxidase (POD) activity observed in certain lines may be linked to the fact that these plants were subjected to environmental stress. Faced with these stress conditions, these lines mobilised tolerance mechanisms by strengthening their cell walls. According to the work of Pandey *et al* (2017), peroxidase (POD) plays an essential role in several vital plant functions, particularly in lignin biosynthesis. The activity of the enzyme phenylalanine ammonia-lyase (PAL) plays a key role in plant defence reactions. PAL is involved in the synthesis of salicylic acid (SA) and other phenolic compounds such as lignins. According to Chen & Clure (2000), these molecules help to strengthen the pectocellulose walls of plant cells. This is an important plant response to various stresses. Catalase (CAT) is also essential for plants. CAT acts by oxidising the cells to eliminate cytotoxic effects. In this case, CAT levels were found to be higher only in the S76A line. This suggests that this line has developed a more efficient enzymatic system to withstand water stress, particularly in terms of light intensity, which can be high. According to studies by Pan *et al* (2020) and Kim *et al* (2020), high concentrations of the enzyme catalase (CAT) give plants a greater capacity to tolerate high light intensity, particularly when they are exposed to water stress. The hydrogen peroxide (H2O2) produced in the plastids is transferred to the peroxisomes thanks to the presence of the CAT enzyme (Bapatla *et al*., 2021). The high CAT content observed in the S76A line suggests that it has more effective detoxification mechanisms to cope with this type of combined stress.

CONCLUSION

This study assessed the physiological and biochemical behaviour of different plant lines (S03, S20, S24, S76A) compared with a control (EV8728). The results showed variable effects, both harmful and beneficial, of the lines on the parameters analysed. These parameters proved to be line-dependent. In terms of photosynthetic pigments, chlorophyll a, chlorophyll b and total chlorophyll levels were higher in the S03 line than in the EV8728 control. The different lines studied showed distinct profiles in terms of phenolic compounds and total sugars. Line S20 accumulated more phenolic compounds. Line S24 showed higher levels of total sugars. In terms of enzyme activity, the enzymes TAL, PAL, PPO, POD and CAT were found to accumulate more in lines S03, S76A, S20 and S24. These accumulations of defence compounds and enzymes in certain lines reveal the implementation of protection and adaptation mechanisms in the face of various environmental aggressions and constraints. Finally, this study showed that the S24 and S76A lines appear to have better physiological and biochemical properties than the control variety EV8728 for coping with changes in soil and climate.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

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